

## Effect of 6, Benzyl amino purine (BAP) on callus production and shoot regeneration in *Bunium persicum* B.Fedtsch

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### ABSTRACT

Various concentrations of BAP were used in order to find out the optimum concentration of this growth substance for callus production as well as for shoot regeneration in *Bunium persicum*. The optimum concentration of BAP for callus production was BAP 1mg/l, in terms of average number of days taken for callus production (6 days), amount of callus produced and percent culture response (100%). However, BAP at a concentration of 4 mg/l was found optimum for shoot regeneration.

### INTRODUCTION

*Bunium persicum* belongs to the family Apiaceae [1]. It is a graminous, dicotyledonous, perennial and self pollinated plant with hermaphrodite flowers. This plant is diploid ( $2n=2x=14$ ) [2] and is commonly known as “Kala Zira” or “Black cumin”. The economic production of *B. persicum* is through seeds (schizocarp fruits) that are used as medicine and spices [3]. Black cumin essential oil is used in pharmaceutical, food sweetening, soft drink, food and hygiene industries [4]. The ripe fruits are used as carminative, lactagogue, diuretic, expectorant, antispasmodic, antiobesity and a valuable spice for flavouring foods [5]. In addition, the essential oil is reported to exhibit significant antioxidative [6], antibacterial [7] and antifungal [8] activities. It also has digestive, anti convulsion and anti-asthma properties and has been reported to increase milk secretion. It is used for genital and diurnary excretion disorders. The plant is also used in treatment of diarrhea, dyspepsia, fever,

flatulence, stomachache, hemorrhoids and as an antihistaminic[9]. The main cause of depletion of this plant has been found to be the thoughtless, improper and unscientific commercial exploitation of seeds for rapid financial gains. The competition for its seeds is so severe that, instead of collecting the ripe seeds, the entire plant is removed even when the seeds are immature[10]. Another major problem encountered in the cultivation of this species is long seed to seed cycle 4-5years [11]. During the present study *B.persicum* has been subjected to *in vitro* studies as it offers an attractive alternative approach for rapid multiplication of such plants.

Plant growth regulators are the substances that are active at very low concentrations and have a regulatory rather than a nutritional role in growth and development of plants. During the present study one such growth adjuvant 6, Benzyl amino purine (BAP) has been used to find its optimum concentration for *in vitro* regeneration of *B. persicum*.

## MATERIALS AND METHODS

During the present study stem segments obtained from *in vitro* raised plants were used as explants. The regenerated *in vitro* plantlets were taken out of the culture flasks aseptically under Laminar air flow hood with the help of sterilized forceps and placed into pre-autoclaved petriplates. The plantlets were cut into 3-5 segments to be used as explants. These explants were inoculated on MS medium fortified with different concentrations of BAP. Cultures were maintained under controlled growth conditions and periodic observations were recorded for callus induction and shoot regeneration. The data was analysed by calculating the standard error.

## RESULTS

*In vitro* raised stem explants were inoculated on MS medium fortified with different concentration of BAP in order to find the

optimum concentration of this growth substance for callus production and shoot regeneration.

Different concentrations of BAP (0.5-2.5 mg/l) were used for callus production from *in vitro* raised stem explant. Callus produced on all the concentrations of BAP (0.5, 1, 1.5, 2 and 2.5 mg/l) used after 8, 6, 11, 8 and 7 average number of days of inoculation respectively with the percent culture response of 90%, 100%, 60%, 40% and 20% respectively (Fig. a, b, c, d and e respectively). From the table 1 it is clear that BAP at a concentration of 1mg/l is most effective for callus production in terms of average number of days taken for callus production (6 days), amount of callus produced and percent culture response (100%). Hence it can be concluded that BAP at a concentration of 1mg/l is optimum for callus production in *B.persicum*.

**Table 1: Optimum concentration of BAP for callus production**

MS medium + concentration of BAP mg/l used	Average number of days taken for callus production	Amount of callus produced	% culture response
0.5mg/l	8	++	90
1mg/l	6	+++	100
1.5mg/l	11	+++	60
2mg/l	8	+	40
2.5mg/l	7	+	20

+ Less amount of callus

++ Medium amount of callus

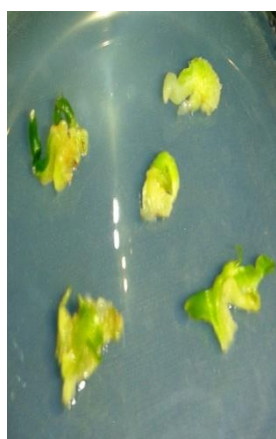
+++ High amount of callus

**Table 2: Optimum concentration of BAP for shoot regeneration**

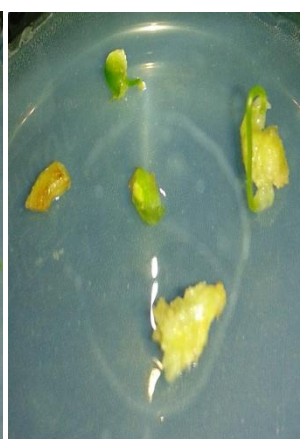
MS medium + Concentration of BAPmg/l used	Average number of days taken for shoot regeneration	Mean number of shoots $\pm$ SE
0.5	20	7.0 $\pm$ 1.11
1	18	3.0 $\pm$ 0.57
1.5	21	6.3 $\pm$ 0.88
2	18	3.3 $\pm$ 0.88
2.5	17	5.3 $\pm$ 0.88
3	11	9.6 $\pm$ 1.20
3.5	31	10.6 $\pm$ 3.20
4	16	13.3 $\pm$ 2.96
4.5	33	12.3 $\pm$ 2.40
5	31	9.6 $\pm$ 2.51



**Fig. a**



**Fig. b**



**Fig.c**



**Fig. d**



**Fig. e**



Fig. f



Fig. g



Fig. h



Fig. i



Fig. j



Fig. k



Fig. l



Fig. m



Fig. n



Fig. o

BAP was used in the concentration range of 0.5- 5mg/l to find its optimum concentration for shoot regeneration after sub-culturing of

callus. BAP at a concentration of 0.5, 1, 1.5, 2, 2.5mg/l regenerated  $7.0 \pm 1.11$ ,  $3.0 \pm 0.57$ ,  $6.3 \pm 0.88$ ,  $3.3 \pm 0.88$ ,  $5.3 \pm 0.88$  mean number of

shoots respectively (Fig. f, g, h, i, j) after 20, 18, 21, 18, 17 average number of days respectively. By increasing the concentration of BAP (3, 3.5, 4 mg/l) shoots regenerated (Fig. k, l, m) after 11, 31, 16 average number of days respectively, with increased mean number of shoots ( $5.3 \pm 0.88$ ,  $9.6 \pm 1.20$ ,  $10.6 \pm 3.20$ ,  $13.3 \pm 2.96$  respectively). Further increase in the concentration of BAP (4.5 and 5mg/l) also regenerated shoots ( Fig. n and o) after 33 and 31 average number of days respectively but the mean number of shoots ( $12.3 \pm 2.40$ ,  $9.6 \pm 2.51$ ) decreased. From Table 2, it is clear that the optimum concentration of BAP for shoot regeneration is BAP 4mg/l at which  $13.3 \pm 2.96$  mean number of shoots regenerated after 16 average number of days of sub-culturing of callus.

## DISCUSSION

The present study was aimed at to find the optimum concentration of BAP for callus induction and shoot regeneration in *B.persicum*. The results obtained have been discussed in the light of existing literature available on effect BAP on *in vitro* regeneration of plants belonging to family Apiaceae. For callus production BAP was used in the concentration range of 0.5- 2.5 mg/l and the optimum concentration was found BAP 1 mg/l on which high amount of callus was produced with 100% culture response. Our results differ from those of Sarkheil *et al.*, (2008) who also used three concentrations of BAP (0.25, 0.5 and 1) for callus induction in *Foeniculum vulgare*, but reported callus induction on medium fortified with BAP (0.5 mg/l) in combination with 2,4-D (0.5 mg/l). Similarly for shoot regeneration BAP was used in the concentration range of 0.5- 5 mg/l and concentration of 4 mg/l was found optimum in terms of mean number of shoots regenerated i.e;  $13.3 \pm 2.96$ . Here again our results are different from the results of Irvani

*et al.*, (2010) who also used BAP in the concentration range of 1-4 mg/l for shoot regeneration of *Dorema ammoniacum*, but they reported best shoot regeneration on medium fortified with BAP (2mg/l) in combination with IBA (0.2 mg/l).

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